

Effect of natural volatile compounds on antioxidant capacity and antioxidant enzymes in raspberries

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Abstract

Changes in antioxidant capacity and antioxidant enzyme activities in raspberries (*Rubus idaeus* L.) treated with methyl jasmonate (MJ), allyl isothiocyanate (AITC), essential oil of *Melaleuca alternifolia* (tea tree oil or TTO), and ethanol (EtOH) were studied. All of the natural volatile compounds tested reduced the severity of decay during storage at 10 °C compared to the control. Most of these natural volatile treatments promoted the antioxidant capacity and antioxidant enzyme activities except AITC treatment. The MJ treatment had the highest antioxidant capacity expressed as oxygen radical absorbance capacity (ORAC) values after storage for 7 and 14 days. Raspberry extract from the MJ treatment also showed the highest activity in all antioxidant enzymes, including superoxide dismutase (SOD), guaiacol peroxidase (G-POD), ascorbate peroxidase (AsA-POD), glutathione peroxidase (GSH-POD), glutathione reductase (GR), monodehydroascorbate reductase (MDAR), and dehydroascorbate reductase (DHAR). Moreover, the MJ treatment showed the highest amount of ascorbate (AsA), dehydroascorbate (DHAsA), reduced glutathione (GSH), and oxidized glutathione (GSSG) compared to the other treatments. Even though AITC showed the best result for decay inhibition among all the treatments, it did not increase the antioxidant capacity or the antioxidant enzyme activities. These results indicate that MJ may increase the resistance of tissues to decay through enhancing their antioxidant system and their free radical scavenging capability, while AITC may retard the decay directly by its antimicrobial properties.

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1. Introduction

Fruit are one of the most important sources of antioxidants, such as carotenoids, phenols, flavonoids, vitamins, and dietary glutathiones. These antioxidants are capable of acting as free radical scavengers, peroxide decomposers, singlet and triplet oxygen quenchers, enzyme inhibitors and synergists (Larson, 1988). Berry fruit, including raspberries, have been reported to contain high phenolic and anthocyanin content (Heinonen et al., 1998; Kähkönen et al., 2001) and have been shown to inhibit both low-density lipoprotein (LDL) and liposome oxidation (Heinonen et al., 1998). Thus, vari-

ous antioxidants found in berry fruit provide significant health benefits.

Several natural volatile compounds including methyl jasmonate (MJ), allyl isothiocyanate (AITC), ethanol (EtOH), and tea tree oil (TTO) have been studied for their effectiveness in maintaining the quality of fruit and vegetables. It has been reported that MJ treatment can reduce the development of chilling injury symptoms in zucchini (Wang and Buta, 1994) and mango (González-Aguilar et al., 2000). In addition, MJ has been shown to suppress fungal growth in grapefruit (Droby et al., 1999), reduce decay and maintain postharvest quality of papayas (González-Aguilar et al., 2003), and inhibit microbial contamination of fresh-cut celery and peppers (Buta and Moline, 1998). AITC is a major flavoring constituent of wasabi (*Eutrema wasabi* Maxim.) and black mustard (*Brassica nigra* Koch) (Ogawa et al., 2000).

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It has been reported that AITC can maintain the postharvest quality of raspberries (Wang, 2003). It was also shown that blue mold (*Penicillium expansum*) in pears was controlled by AITC vapor treatment (Mari et al., 2002). Furthermore, the bactericidal activity of AITC against pathogens on iceberg lettuce, apples, and tomatoes has also been reported (Lin et al., 2000). Ethanol treatment was shown to be effective in controlling fungal decay of table grapes (Lichter et al., 2002). The efficacy of EtOH has also been demonstrated in inhibiting decay on lemons (Smilanick et al., 1995), and in combination with a hot water treatment on sweet cherry in retarding postharvest diseases (Karabulut et al., 2004). In addition, EtOH treatment delays senescence in broccoli florets (Suzuki et al., 2004). The essential oil from *Melaleuca alternifolia*, or TTO, has shown a high level of antifungal activity in various crops (Bishop and Thornton, 1997). However, there is little information on the effect of natural volatiles on antioxidant capacity and antioxidant enzyme activities.

The purpose of this study was to determine the changes in the activities of antioxidant enzymes and antioxidant capacity in raspberries treated with these natural volatile compounds. The effect of natural volatile compounds on decay in raspberry was also evaluated.

2. Materials and methods

2.1. Chemicals

Ascorbate, chlorogenic acid, β -carotene, histidine, hydrogen peroxide, hydroxylamine hydrochloride, *N,N*-dimethyl-*p*-nitrosoaniline, xanthine, xanthine oxidase, dithiothreitol (DTT), glutathione (oxidized form), glutathione (GSH, reduced form), glutathione reductase, guaiacol, β -nicotinamide adenine dinucleotide (β -NADH, reduced form), β -nicotinamide adenine dinucleotide phosphate (β -NADPH, reduced form), nitro blue tetrazolium (NBT), Chelex 100, and FeSO_4 were purchased from Sigma Chemical Co. (St. Louis, MO). Trichloroacetic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and α -tocopherol were purchased from Aldrich (Milwaukee, WI). 2',2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA, Inc. (Richmond, VA).

2.2. Fruit sample handling and treatments with natural volatile compounds

Raspberries (*Rubus idaeus* L., cv. Estes) used in this study were grown at a farm near Beltsville, Maryland, USA, and were hand-harvested at a commercially mature stage, sorted to eliminate damaged, shriveled, and unripe fruit, and selected for uniform size and color. Selected berries were randomized and used for the experiments. Fifty fruit were placed into one liter polystyrene containers with snap-on lids. Volatile

compounds used in this study include methyl jasmonate ($22.4 \mu\text{l l}^{-1}$), allyl isothiocyanate ($5 \mu\text{l l}^{-1}$), essential oil of *Melaleuca alternifolia* (tea tree oil, $100 \mu\text{l l}^{-1}$), and ethanol ($200 \mu\text{l l}^{-1}$). The specified volume of each volatile compound was spotted onto a piece of filter paper which was subsequently hung inside the plastic containers just before the lids were closed. The volatile compounds were allowed to vaporize inside the containers spontaneously at 20°C for 16 h. The containers were then stored at 10°C . Three containers were used for each treatment. Control samples were handled similarly but omitting the volatile treatment. Samples were taken initially and at 7 and 14 days during storage. The samples were then frozen in liquid nitrogen and then stored at -80°C until assayed for antioxidant capacity, antioxidant enzyme activities, and non-enzyme components. The development of decay was evaluated every other day and the severity was expressed as percent of fruit showing fungal symptoms.

2.3. Total anthocyanin and total phenolic content

Raspberries were extracted with 80% acetone containing 0.2% formic acid using a Polytron (Brinkmann Instruments, Inc., Westbury, NY). The homogenized samples from the acetone extracts were then centrifuged at $14,000 \times g$ for 20 min at 4°C . The supernatants were transferred to vials, stored at -80°C , and later used for anthocyanin, total phenolic, and antioxidant analysis.

Total anthocyanin content in fruit extracts was determined using the pH differential method (Cheng and Breen, 1991). Absorbance was measured in a spectrophotometer (Shimadzu UV-1601, Shimadzu Scientific Instruments, Columbia, MD) at 510 and 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{\text{pH } 1.0} - (A_{510} - A_{700})_{\text{pH } 4.5}]$ with a molar extinction coefficient of cyanidin-3-galactoside. Total soluble phenolics in the fruit extracts were determined with Folin-Ciocalteu reagent by the method of Slinkard and Singleton (1977) using gallic acid as a standard.

2.4. Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was carried out using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system and a microplate fluorescence reader (Huang et al., 2002). The automated sample preparation was performed using a Precision 2000 instrument. The sample series dilution sequence was programmed and controlled by the precision power software. The ORAC values were determined by calculating the net area under the curve (AUC) of the standards and samples. The standard curve was obtained by plotting Trolox concentrations against the average net AUC of the two measurements for each concentration. Final ORAC values were calculated using the regression equation between Trolox concentration and the net AUC.

2.5. Antioxidant enzyme measurements

2.5.1. Glutathione-peroxidase (GSH-POD, EC 1.11.1.9) and glutathione reductase (GR, EC 1.6.4.2)

Fruit tissue (4 g fresh weight) was homogenized in 4 ml 0.1 M Tris–HCl buffer (pH 7.8) containing 2 mM EDTA–Na, and 2 mM dithiothreitol. The homogenate was centrifuged at $20,000 \times g$ for 30 min at 4 °C, and the supernatant was used for the GSH-POD and GR assays.

GSH-POD activity was determined using the method of Tappel (1978) with a slight modification. The reaction mixture contained 0.1 M Tris–HCl buffer (pH 8.0), 0.4 mM EDTA, 1.0 mM NaN_3 , 1.0 mM H_2O_2 , 1.0 mM glutathione (GSH), 0.15 mM NADPH, 1 unit of glutathione reductase, and 100 μl enzyme extract. The total reaction volume was 1.0 ml. The reaction was started by adding H_2O_2 . GSH-POD activity was determined by the rate of NADPH oxidation at 340 nm via a spectrophotometer.

GR activity was assayed according to Smith et al. (1988). The activity of GR was determined by monitoring glutathione-dependent oxidation of NADPH at 340 nm. The reaction was started by adding GSSG (oxidized glutathione) and the rate of oxidation was calculated using the extinction coefficient of NADPH ($6.22 \text{ mM}^{-1} \text{ cm}^{-1}$).

2.5.2. Superoxide dismutase (SOD, EC 1.15.1.1)

Fruit tissue (4 g) was pulverized with a cold mortar and pestle with 4 ml K-phosphate buffer (0.1 M, pH 7.3) containing 1 mM EDTA, 2 mM DTT. The homogenate was strained through four layers of miracloth (Calbiochem, La Jolla, CA) and centrifuged at $12,000 \times g$ for 10 min at 4 °C. The supernatant was used for assaying the SOD enzyme activity. Total SOD activity was assayed photochemically (Monk et al., 1987; Thayer, 1990). One unit of SOD was defined as the amount of enzyme which produced a 50% inhibition of NBT reduction under assay conditions.

2.5.3. Ascorbate peroxidase (AsA-POD, EC 1.11.1.11) and guaiacol peroxidase (G-POD, EC 1.11.1.7)

Fruit tissue (4 g) was pulverized with a cold mortar and pestle with 4 ml K-phosphate buffer (0.1 M, pH 7.3) containing 1 mM EDTA, and 2 mM DTT. The homogenate was centrifuged at $12,000 \times g$ for 10 min at 4 °C. The supernatant was used for the AsA-POD and G-POD assays.

AsA-POD activity was assayed according to the method of Amako et al. (1994). The reaction was started by adding H_2O_2 . The G-POD assay mixture contained 0.1 M phosphate buffer (pH 6.1), 4 mM guaiacol as donor, 3 mM H_2O_2 as substrate, and 1.0 ml crude enzyme extract. The total reaction volume was 3.0 ml. The rate of change in absorbance at 420 nm was measured, and the level of enzyme activity was expressed as the difference in absorbance (OD).

2.5.4. Dehydroascorbate reductase (DHAR, EC 1.8.5.1)

DHAR activity was assayed by measuring the rate of NADPH oxidation at 340 nm (Shigeoka et al., 1980). The

reaction mixture contained 50 mM potassium phosphate (pH 6.1), 0.2 mM NADPH, 2.5 mM dehydroascorbate, 2.5 mM glutathione, 0.6 unit glutathione reductase (from spinach, EC 1.6.4.2) and 0.1 ml of diluted fruit juice (2 ml juice was diluted with 2 ml 50 mM potassium phosphate, pH 6.1). The reaction was started by adding dehydroascorbate.

2.5.5. Monodehydroascorbate reductase (MDAR, EC 1.6.5.4)

MDAR activity was assayed by measuring the rate of NADH oxidation at 340 nm (Nakagawara and Sagisaka, 1984). The reaction mixture contained 50 mM K-phosphate buffer (pH 7.3), 0.2 mM NADH, 1.0 mM ascorbate, 1.0 unit of ascorbate oxidase and 0.1 ml of 50 mM K-phosphate buffer (pH 7.3) diluted fruit juice (2 to 1 dilution) in a total volume of 1.0 ml. The reaction was started by adding ascorbate oxidase (from *Cucurbita*, EC 1.10.3.3).

2.6. Non-enzyme component measurements

2.6.1. Determination of ascorbate (AsA) and dehydroascorbate (DHAsA)

Fruit samples of 4 g were homogenized with a cold mortar and pestle using 8 ml ice-cooled 5% trichloroacetic acid (TCA). The homogenate was filtered through four layers of miracloth and centrifuged at $16,000 \times g$ for 10 min at 4 °C. The supernatant was used for the AsA and total AsA assays. AsA and DHAsA were determined using the methods of Arakawa et al. (1981). Total AsA (AsA plus DHAsA) was determined through a reduction of DHAsA to AsA by dithiothreitol. DHAsA concentrations were estimated from the difference of “total AsA” and “AsA” concentrations. A standard curve in the range 0–10 μmol AsA or DHAsA was used.

2.6.2. Glutathione (GSH) and oxidized glutathione (GSSG)

Triplicate strawberry fruit samples of 4 g were homogenized in 8.0 ml ice-cold, degassed 7.57 mM sodium ascorbate solution with chilled mortar and pestle under N_2 at 0 °C. The homogenate was filtered through four layers of miracloth and centrifuged at $30,000 \times g$ for 15 min at 0 °C. The supernatant was deproteinized in glass test tubes by incubation in a water bath at 100 °C for 3 min and then centrifuged at $15,000 \times g$ for 15 min at 0 °C. The supernatants were used for the GSH and GSSG assays. GSH and GSSG were assayed using the method described by Castillo and Greppin (1988). GSSG was determined by subtraction of GSH from total glutathione.

3. Results

3.1. Total anthocyanin and total phenolic content

Total anthocyanin and total phenolic content from different raspberry extract treatments are shown in Fig. 1A–D.

Total anthocyanin content and total phenolic content range from 0.69 to 1.68 and 7.81 to 9.91 g kg⁻¹, respectively. Raspberry extract from the MJ treatment yield the highest total anthocyanin and total phenolic content followed by the EtOH and TTO treatment. The AITC treatment has the lowest total anthocyanin and total phenolic content. Total anthocyanin and total phenolic content from raspberry extract for every treatment decreased when stored for 14 days.

3.2. ORAC values

Antioxidant capacity was expressed as an ORAC value in the various raspberry extract treatments (Fig. 1E and F). The ORAC values of raspberry extract range from 53.52 to 83.13 mmol kg⁻¹ after 7 days of storage and from 50.32 to 81.71 mmol kg⁻¹ fresh berries after 14 days of storage. The

extract from both the 7 and 14 days of storage for the MJ treatment had the highest ORAC values followed by the extracts from EtOH and TTO treatment, whereas the AITC treatment had the lowest ORAC values.

3.3. Antioxidant enzymes

3.3.1. Glutathione-peroxidase (EC 1.11.1.9) and glutathione reductase (EC 1.6.4.2)

GSH-POD and GR activities of raspberry extracts from 7 and 14 days of storage varied among treatments as shown in Fig. 2A–D. Raspberry extract from MJ treatment had the highest activities, while those from EtOH and TTO treatments had lower activities, and samples from the AITC treatment had the lowest activities for GSH-POD and GR. GSH-POD and GR activities decreased with longer storage duration.

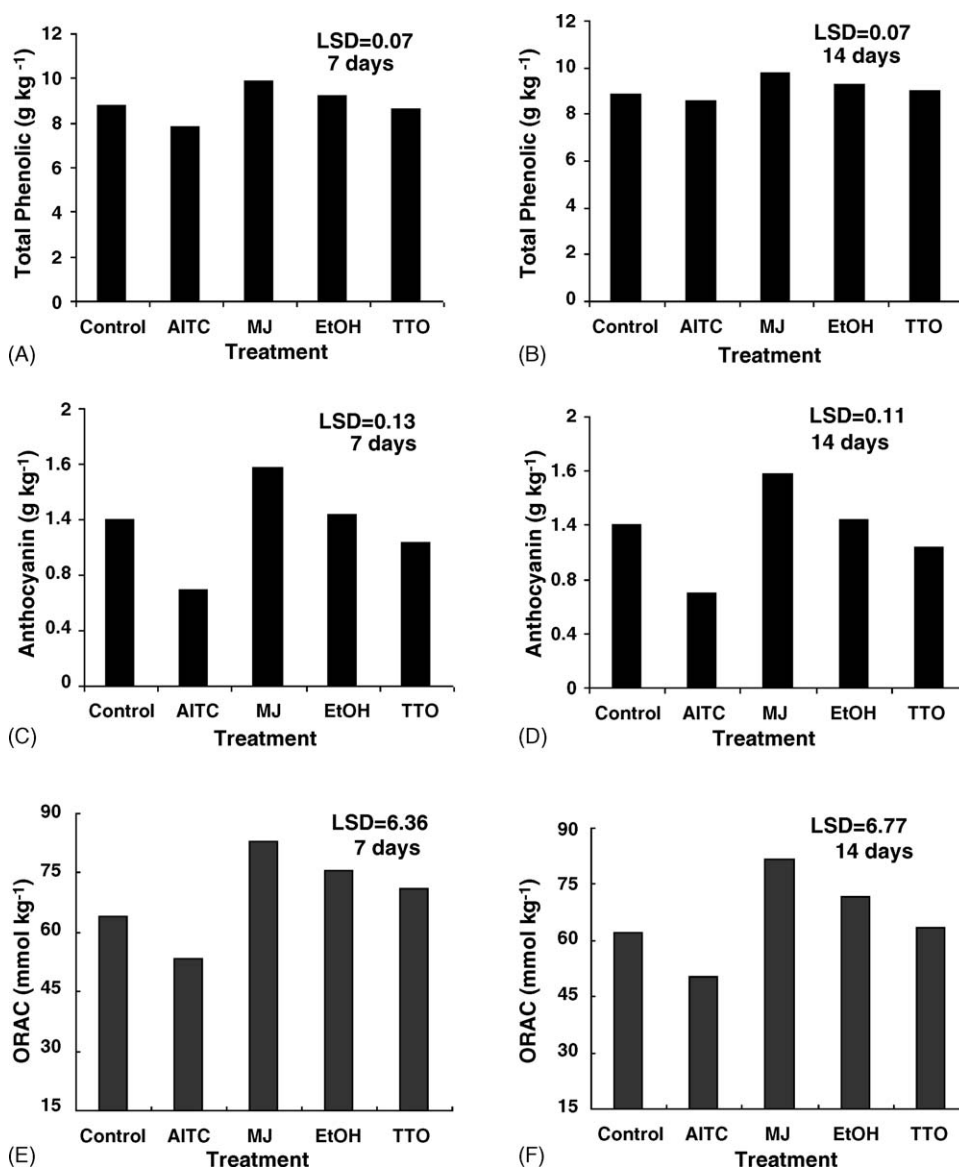


Fig. 1. Total phenolic content, anthocyanin content, and ORAC value in raspberry fruit treated with various natural volatile compounds and stored for 7 and 14 days at 10 °C.

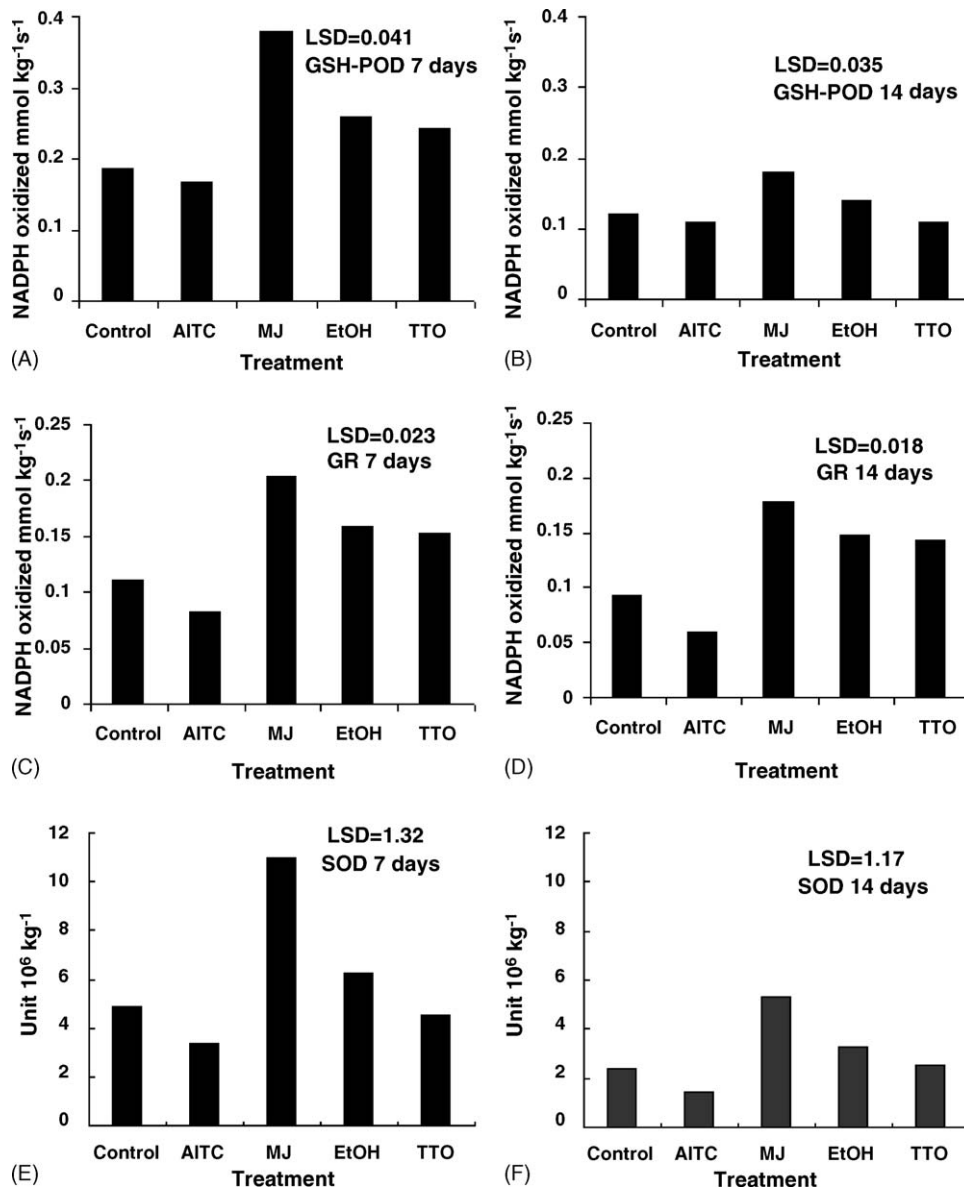


Fig. 2. Glutathione peroxidase (GSH-POD), glutathione reductase (GR), and superoxide dismutase (SOD) activity in raspberry fruit treated with various natural volatile compounds and stored for 7 and 14 days at 10 °C.

3.3.2. Superoxide dismutase (EC 1.15.1.1)

Raspberry extracts taken after 7 days of storage had higher SOD activities than those taken after 14 days of storage as shown in Fig. 2E and F. SOD activities decreased with storage time. Raspberry extract from the MJ treatment had the highest activity, followed by the extracts from EtOH, TTO, and AITC in both 7 and 14 days of storage.

3.3.3. Ascorbate peroxidase (EC 1.11.1.11) and guaiacol peroxidase (EC 1.11.1.7)

Both AsA-POD and G-POD had the highest activities in raspberry extracts of the MJ treatment followed by EtOH and TTO treatments (Fig. 3). Raspberry extracts from the AITC

treatment consistently had the lowest activities for AsA-POD and G-POD. AsA-POD and G-POD activities also decreased during storage.

3.3.4. Monodehydroascorbate reductase (EC 1.6.5.4) and dehydroascorbate reductase (EC 1.8.5.1)

MDAR and DHAR activities for raspberry extract varied among treatments. Raspberry extracts from MJ treatment stored for 7 days had the highest MDAR and DHAR activities (Fig. 4A–D). Raspberry extracts from AITC treatment after 14 days of storage had the lowest activities of both MDAR and DHAR. During storage from 7 to 14 days, MDAR and DHAR activity decreased in all treatments.

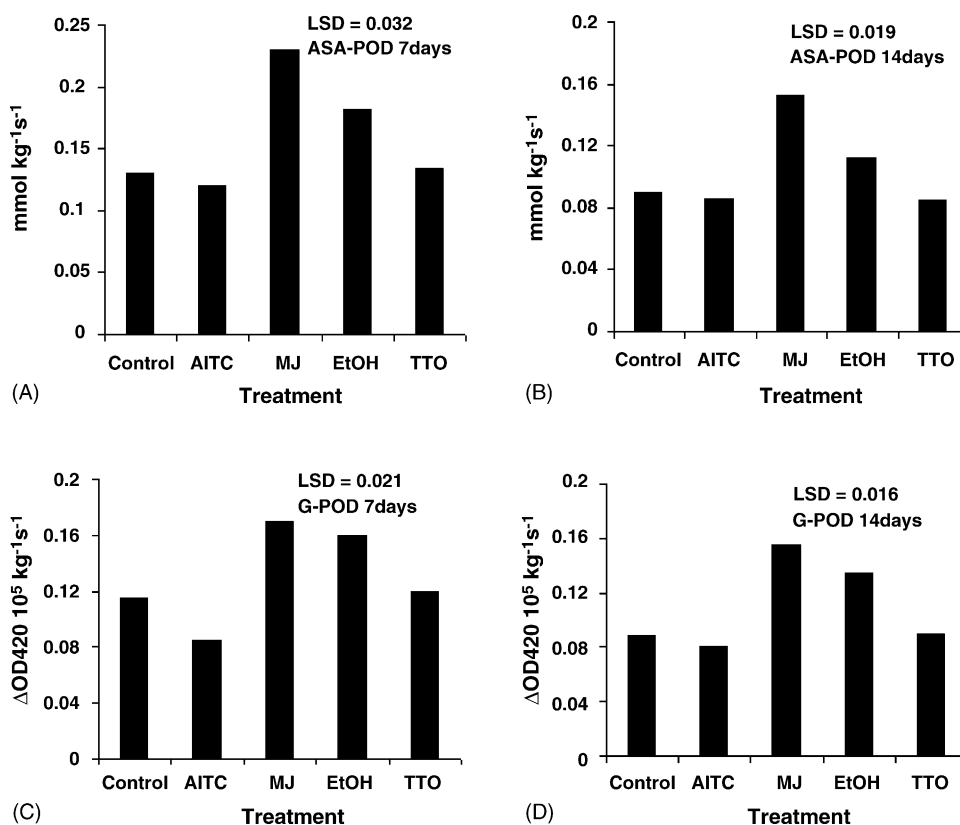


Fig. 3. Ascorbate peroxidase (AsA-POD) and guaiacol peroxidase (G-POD) activity in raspberry fruit treated with various natural volatile compounds and stored for 7 and 14 days at 10 °C.

3.4. Non-enzyme components

3.4.1. Ascorbate and dehydroascorbate

The highest amount of AsA and DHAsA was observed in the extracts from raspberry stored for 7 days from the MJ treatment with 1.1 and 0.17 mmol kg^{-1} , respectively (Fig. 5). The lowest levels of AsA and DHAsA were found in the AITC treatment stored for 14 days with 0.61 and 0.1 mmol kg^{-1} , respectively.

3.4.2. Reduced glutathione and oxidized glutathione

The amounts of GSH varied from 31.64 to 54.64 $\mu\text{mol kg}^{-1}$ fresh berries and GSSG ranged from 7.47 to 20.16 $\mu\text{mol kg}^{-1}$ fresh weights as shown in Fig. 6A–D. The highest values of GSH and GSSG were found in the MJ treatment, while the lowest amounts of these components were observed in the AITC treatment.

3.5. Decay evaluation

All natural volatile compounds tested in this study reduced decay in raspberries during storage at 10 °C (Fig. 7). The most effective treatment was AITC, followed by MJ, EtOH, and then TTO. While the control samples started to show mold growth by the 4th day, AITC treated raspberries did not develop any fungal decay until after 12 days. The MJ, EtOH,

and TTO treatments also delayed the onset and the severity of decay, but to a lesser extent.

4. Discussion

Several previous studies have shown that berries are a good source of natural antioxidants (Wang et al., 1996; Heinonen et al., 1998). These natural plant antioxidants include phenolic compounds and anthocyanins (Wang and Lin, 2000). Phenolic compounds in fruit and vegetables may produce beneficial effects by scavenging free radicals (Chun et al., 2003). Thus, phenolic compounds may help protect cells against the oxidative damage caused by free radicals (Wada and Ou, 2002). In our study, total phenolic and total anthocyanin content were variable in raspberry extracts treated with the different natural volatile compounds. Raspberries stored at 10 °C for 7 days had a higher total phenolic content than raspberry stored for 14 days. This decrease with the storage duration occurred in treatments with MJ, EtOH, and TTO. In contrast, raspberry extracts from the AITC treatment increased in total phenolic content when stored for 14 days. Raspberries treated with AITC also had the best control of decay after 14 days of storage. These data indicate that the increased level of total phenols may play a part in the suppression of fungal growth in the AITC treat tissues. The strong antimicrobial activity of

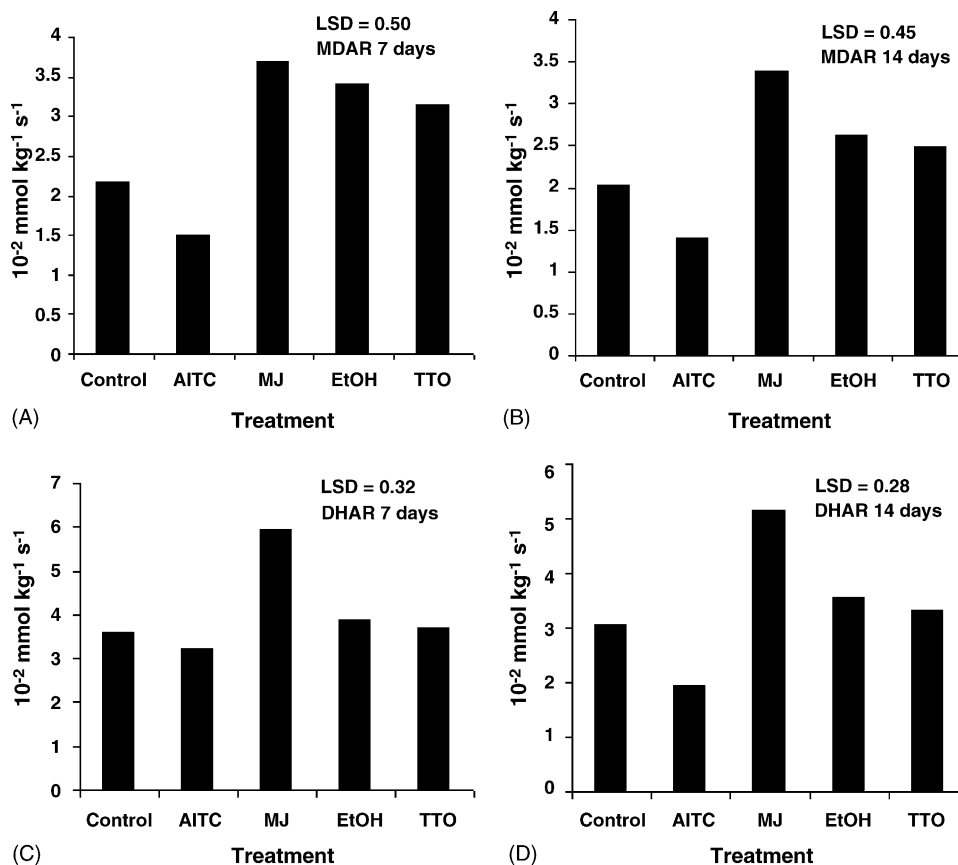


Fig. 4. Monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR) activity in raspberry fruit treated with various natural volatile compounds and stored for 7 and 14 days at 10 °C.

AITC was possibly also responsible for the low percentage of decay in raspberries in this study. In a previous study, it was shown that the amount of total phenolic content in raspberries depends on the seasonal variation, time of harvesting, and the length of frozen storage (Ancos de et al., 2000). Kähkönen et al. (2001) reported that total phenolic content from various raspberries cultivars differed according to dry matter, and berries grown in different locations and seasons also had an effect on total phenolic content. Our study showed that postharvest treatments with different natural volatile compounds could also produce different patterns in the change of phenolic content during storage.

Anthocyanins are a group of phenolic compounds responsible for the red-blue color of many fruit and vegetables, and provide beneficial effects to human health (García-Alonso et al., 2004). The extracts of raspberries treated with MJ had the highest anthocyanin content among all the treatments after both 7 and 14 days of storage. It is probable that MJ enhances the production of total phenolic compound and anthocyanin content in our study and promotes the defense mechanism in raspberries. Raspberries treated with MJ show less decay than all other treatments except AITC. MJ vapor has also been reported to reduce bacterial colonies on celery stalks after one week of storage (Buta and Moline, 1998). Raspberry extracts from the MJ treatment also had the highest

ORAC values when compared with the other treatments. The result was similar to a previous study by Wang (2003).

Vitamin C, including AsA and DHAsA, is one of the most important nutritional quality factors in many horticultural crops and has many main biological activities in the human body (Lee and Kader, 2000). In addition, the most important reducing substrate for H_2O_2 detoxification in plant cells is ascorbate (Mehlhorn et al., 1996). AsA is the principal active form while DHAsA is an oxidation product that also exhibits biological activity. DHAsA can convert to AsA in the human body so it is important to determine both AsA and DHAsA concentrations (Wills et al., 1984). In our study, AsA in all treatments decreased when stored for long periods of time (Fig. 2). Raspberry extracts from the MJ treatment had the highest AsA content after 7 days of storage, and decreased slightly when stored for 14 days. The amount of DHAsA in raspberries treated with the different volatile compounds was varied. The MJ treatment still had the highest amount of DHAsA. When comparing between 7 and 14 days of storage, there was not a significant difference in DHAsA content. DHAsA in some fruit and vegetables increase during storage (Wills et al., 1984). DHAsA will recycle to AsA by dehydroascorbate reductase, while monodehydroascorbate (MDA), a primary product of ascorbate peroxidase can return into the ascorbate pool by monodehydroascorbate reductase

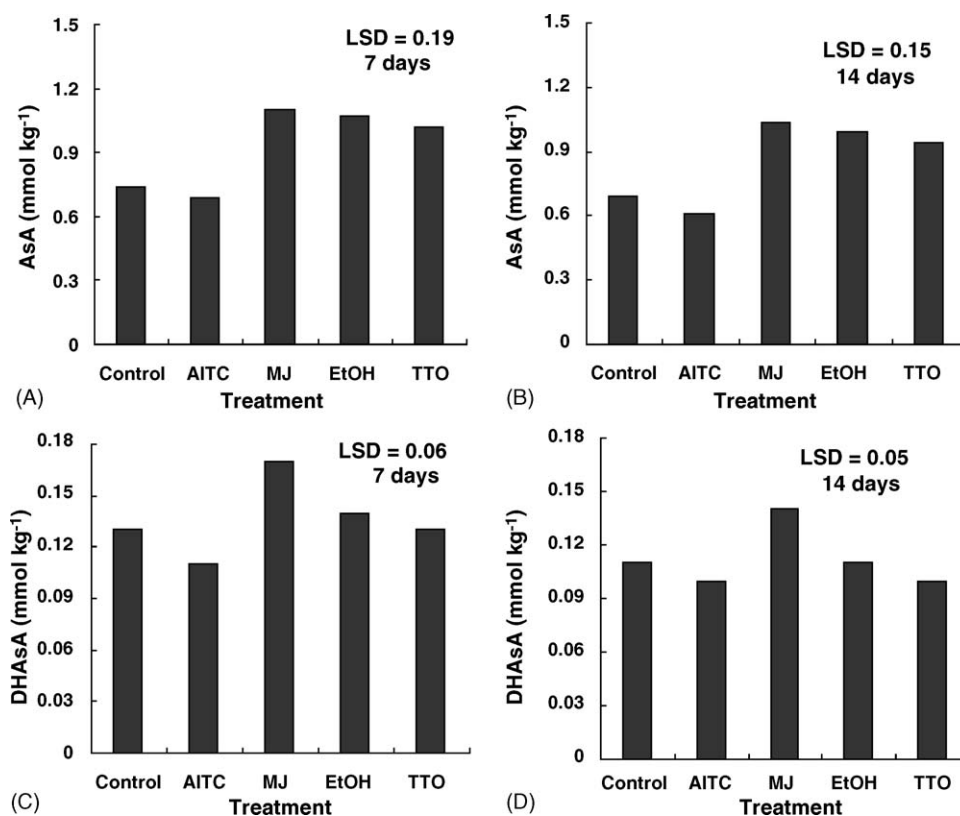


Fig. 5. Ascorbate (AsA) and dehydroascorbate (DHAsA) content in raspberry fruit treated with various natural volatile compounds and stored for 7 and 14 days at 10 °C.

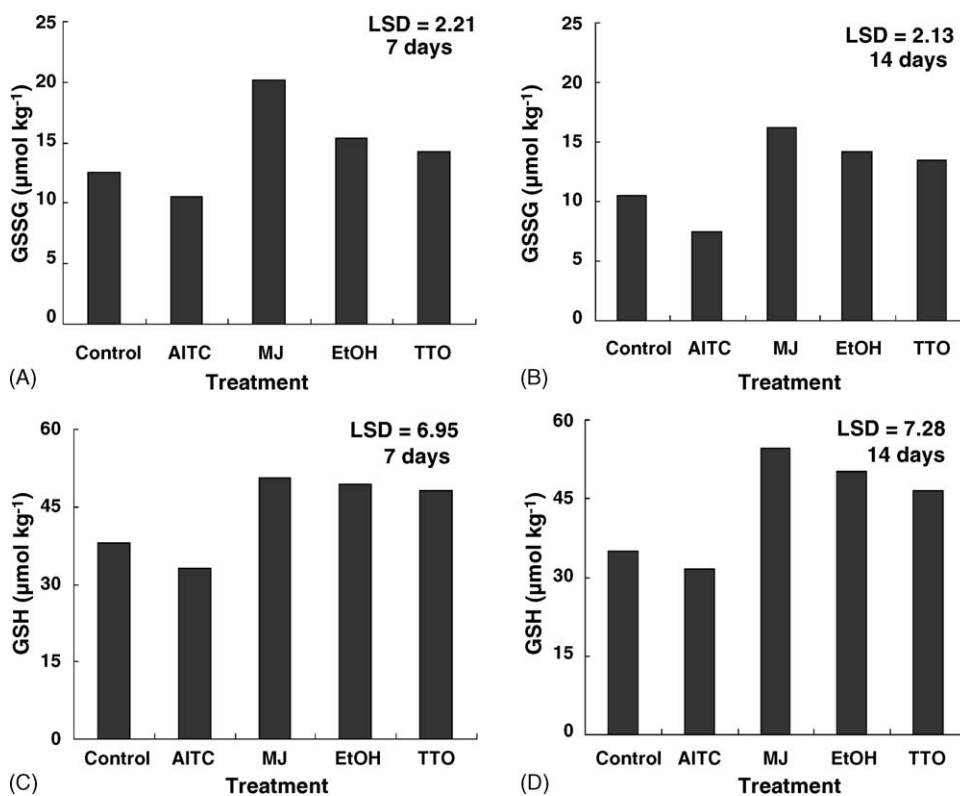


Fig. 6. Reduced glutathione (GSH) and oxidized glutathione (GSSG) content in raspberry fruit treated with various natural volatile compounds and stored for 7 and 14 days at 10 °C.

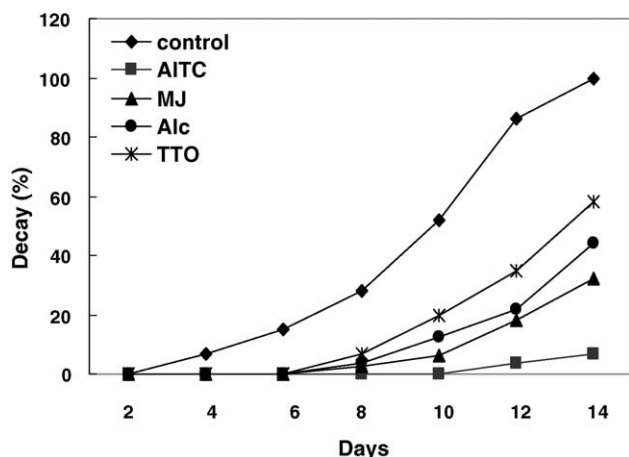


Fig. 7. The effect of various natural volatile treatments on decay of raspberry fruit during storage at 10 °C.

(Morell et al., 1997). In our study, the data showed that MDAR and DHAR were highest in extracts from raspberries treated with MJ after storage for 7 days. The decrease in MDAR and DHAR activities when stored for long periods of time resulted in the decrease of AsA and DHAsA content.

The reduced form of glutathione, GSH, is the major non-protein thiol in most plant species. GSH has an important function in maintaining cellular redox status (Rennenberg, 1980). The primary oxidation product of GSH is its disulfide, GSSG, which can be reduced back to GSH by glutathione reductase at the expense of NADPH (Ric de Vos et al., 1994). In this study, both GR and GSH-POD activities decreased in raspberries stored for 14 days and the MJ treatment had the highest GR and GSH-POD activities. The activity of GSH-POD is dependent on the availability of the reduced ascorbate and GSH that are maintained by enzymes, such as GR, DHAR, and MDHAR using NADPH as an electron donor (Roxas et al., 2000).

Guaiacol peroxidase and ascorbate peroxidase are peroxidase enzymes that are found in animal, plant and microorganism tissues, and which can catalyze oxidoreduction between hydrogen peroxide (H_2O_2) and various reductants (Hiraga et al., 2001). AsA-POD, involved in the detoxification of H_2O_2 , uses two molecules of ascorbate to reduce H_2O_2 to water (Noctor and Foyer, 1998). The different natural volatile compounds have varying effects on G-POD and AsA-POD activities in raspberries. The MJ treatment showed the highest activities in both G-POD and AsA-POD, while the AITC treatment had the lowest activities.

Superoxide dismutases (SOD), a class of metal-containing proteins, catalyze the dismutation reaction of superoxide radical anions into H_2O_2 and molecular oxygen (Scandalios, 1993). There are three different types of SOD categorized by their metal cofactor: Cu/Zn (Cu/Zn-SOD), Mn (Mn-SOD), and Fe (Fe-SOD) (McKersie et al., 1993). However, in this study we determined only the total SOD activity in the raspberries treatments. The result showed that the MJ treatment

had the highest SOD activity, while the AITC treatment had the lowest activity throughout storage, and SOD activities decreased during storage in all treatments.

In conclusion, this study shows that MJ treatment consistently produced higher antioxidant capacity and antioxidant enzyme activity in raspberries than other treatments, whereas the AITC treatment invariably had the lowest values of these antioxidants and enzyme activities. While both MJ and AITC proved to be the two most effective natural volatile compounds tested in reducing decay in raspberries, they seem to exert their effects through different mechanisms. MJ may enhance the resistance of tissues to decay by heightening their antioxidant system and free radical scavenging capability, while AITC may retard decay development by suppressing the decrease of phenolic content and inhibiting microbial growth.

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